

#5

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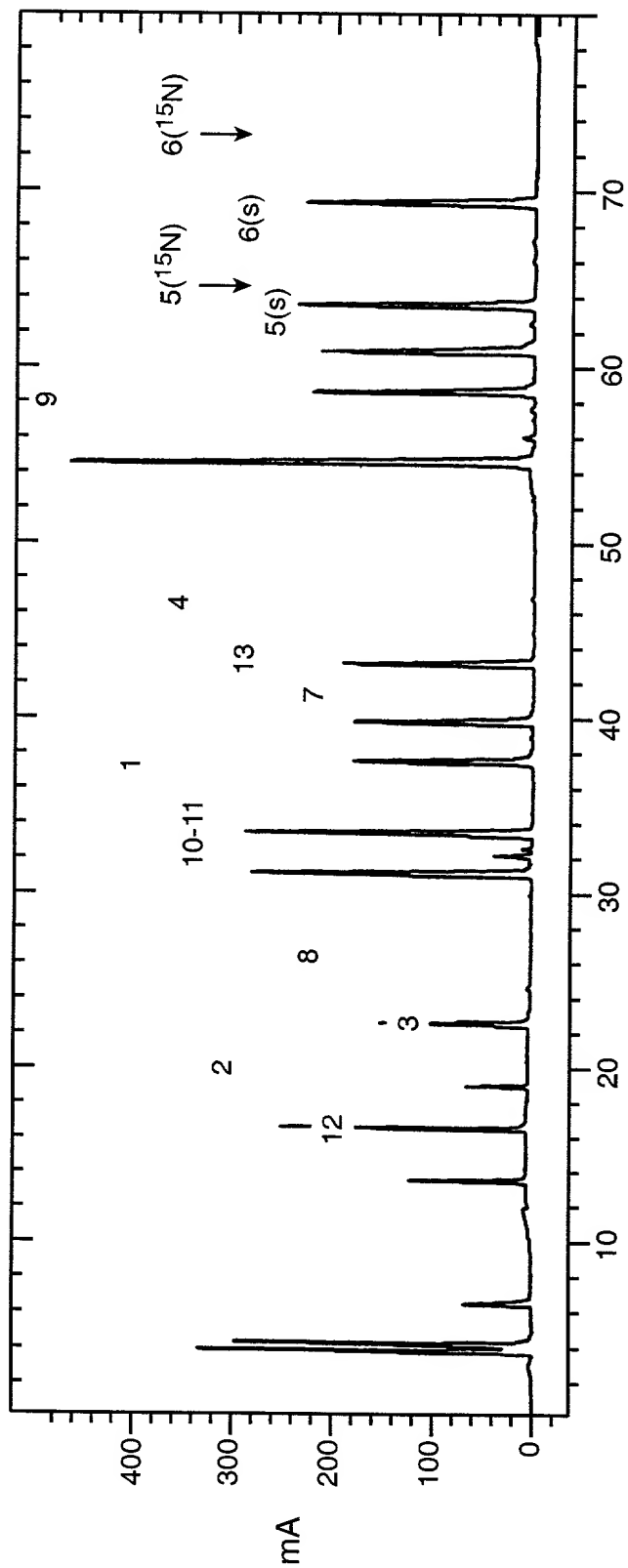


FIG. 1

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FIG._2A

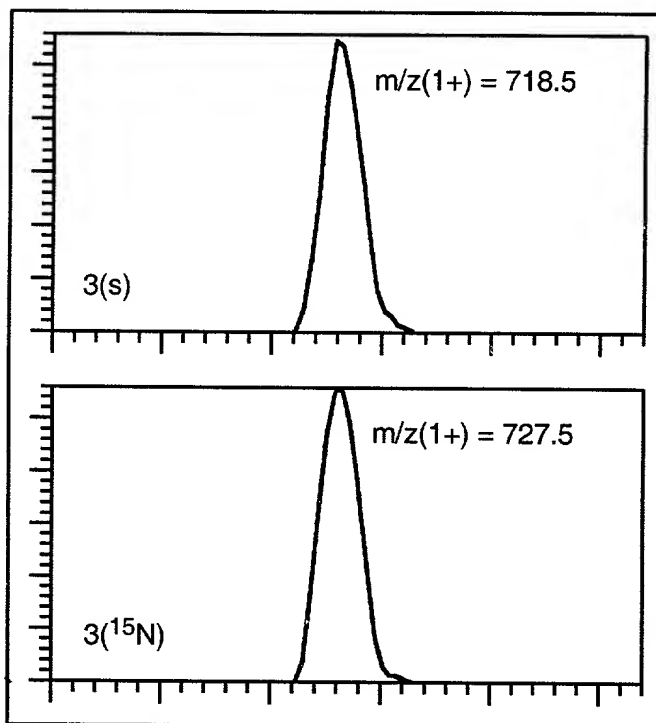
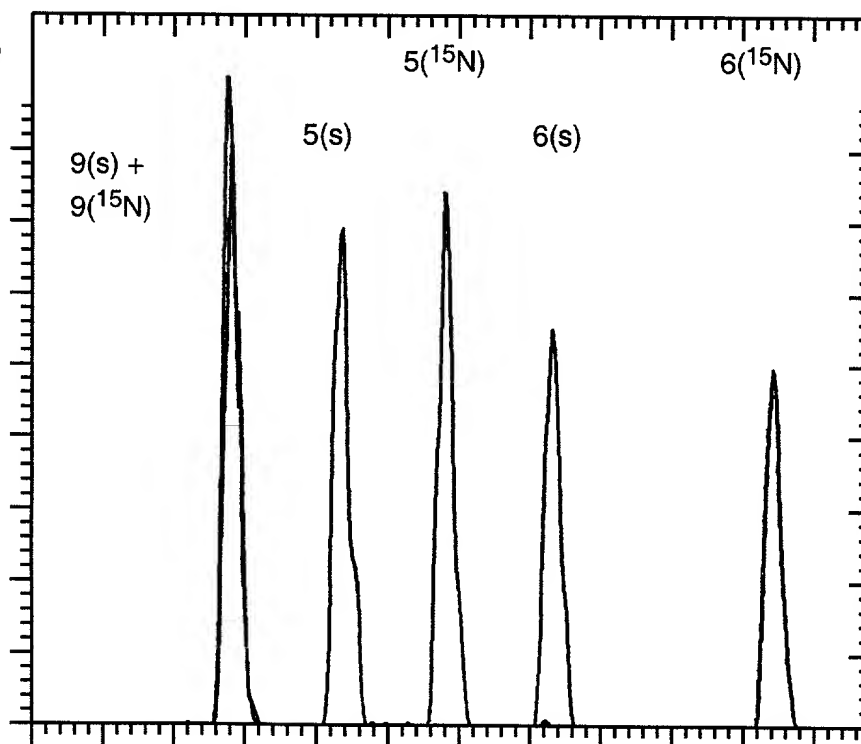


FIG._2B



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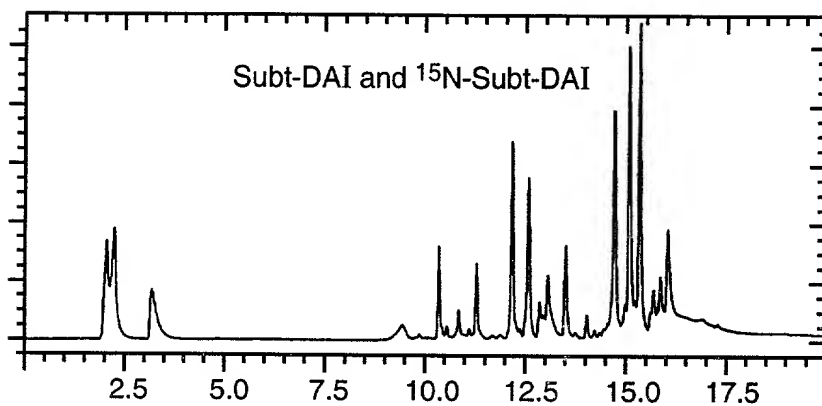


FIG._3

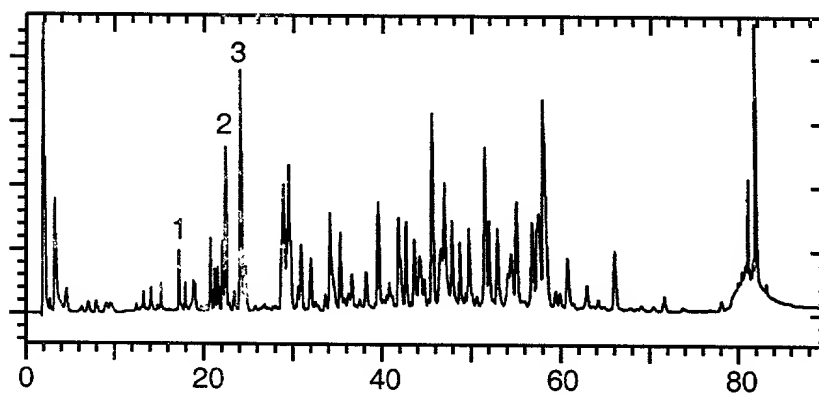


FIG._4A

FIG._4B

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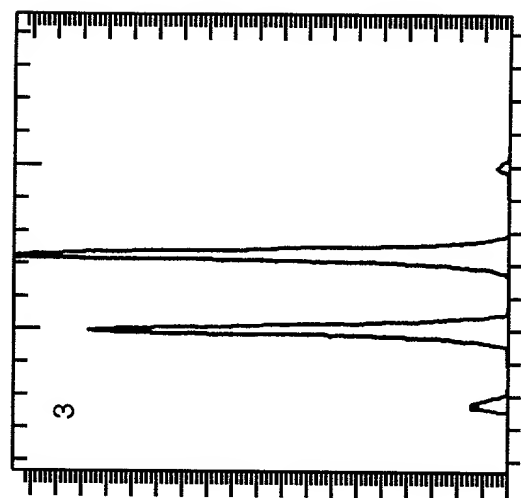


FIG..5(3)

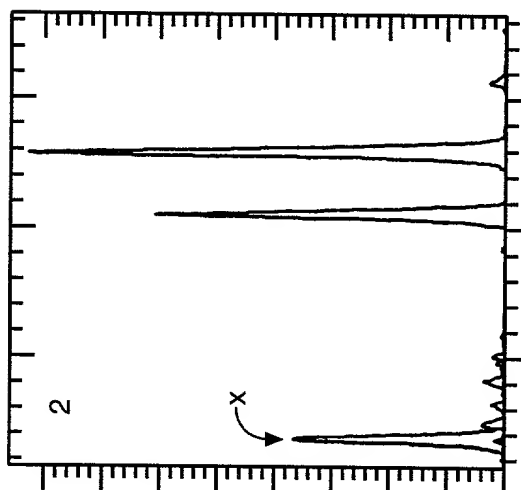


FIG..5(2)

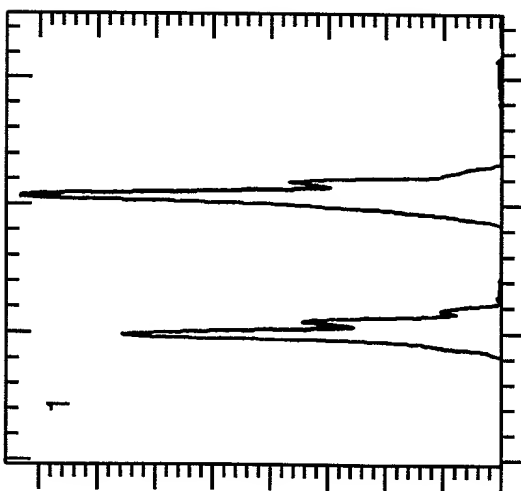


FIG..5(1)

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Pep #	Sequence	m/z (wt)	m/z (¹⁵ N)	TIC Peak Area Ratio	UV Peak Area Ratio
1	AQSVPWGISR	1100.58(1+)	1115.53(1+)	1.013	
2	VQAPAAHNR	482.25(2+)	490.23(2+)	1.028	
3	GLTGSVK	718.40(1+)	727.38(1+)	1.033	
4	VAVLDTGISTHPDLNR	911.49(2+)	922.45(2+)	0.997	
5	GGASFVPEPSTQDGNHGHGTHVAGTIAALDINSIGVLGVAPSAELYAVK	1531.09(3+)	1549.71(3+)	1.049	
5 (subtilisin)	N	1530.77(3+)	-		0.981
6	VLGASGSGAIISSIAQGLEWAGNNGMHVANLSLGSPSPSATLEQAVNSATSR	1642.14	1663.08(3+)	0.979	
6 (subtilisin)	SV	1642.80(3+)	-		1.003
7	GVLVVAASGNSGAGSISYPAR	967.51(2+)	979.47(2+)	1.042	
8	YANAMAVGATDQNNR	855.38(2+)	867.34(2+)	0.971	
9	ASFSQYGAGLDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVK	1600.46(3+)	1619.07(3+)	1.010	
10-11	QK NPSWSNVQIR	729.38(2+)	739.35(2+)	1.044	
12	NHLK	511.29(1+)	519.27(1+)	1.021	
13	NTATSLGSTNLYGSGLVNAEAAATR	1185.08(2+)	1200.04(2+)	1.028	

average peak area ratio: 1.018 ±2.5%

average peak area ratio: 0.992 ±1.6%

average ratio of both methods: 1.005 ±1.3%

intended ratio: 1.000

FIG._6

Table II. Ratio of Concentration and Catalytic Activity (Conversion Factor) of 13 Variants Generated from Subtilisin-DAI and Expressed in Microtiter Plates¹

Variant	Conversion by Peptide Mapping with ¹⁵ N-Internal Protein Standard	
	EL3.16	OS36.7
Clone 1	0.035	0.015
Clone 2	0.037	0.014
Clone 3	0.035	0.015
Clone 4	0.038	0.014
Clone 5	0.038	0.014
Conversion by MBI Titration		
Clone 1	0.036	0.015
		0.020

FIG. 7

¹Clones within the three groups, EL3.16, OS36.7, and EL3.17 had the same sequence. Activity was measured by the suc-AAPF-pNA assay (Hsia et al., 1996). The concentration was measured by the peptide mapping method with ¹⁵N-labeled subtilisin-DAI as internal standard. The range of concentrations was 2 to 5 µg·ml⁻¹. The conversion factor was verified by an active site titration with a mung bean inhibitor (MBI) solution calibrated on the same plate with a previously calibrated solution of subtilisin-DAI (Hsia et al., 1996).